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Antibiotic Susceptibility and Plasmid Profile of *Escherichia coli* from Door Handles in Two Tertiary Institutions in Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOE designed the study and co-ordinated the research. Authors PAT and SAM helped with sample collection and laboratory analysis. Author PAT searched for literatures and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study is aimed at isolation, antibiotic susceptibility and plasmid study of *Escherichia coli* isolates from door handles in the study location.
Study Design: Cross-sectional study.
Place and Duration of Study: This study was conducted in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa, both in Nasarawa state, Nigeria between March 2016 to October 2016.
Methodology: A total of 200 door handles (100 each from the two locations) were sampled and screened for the presence of *E. coli.* Antibiotics susceptibility study, Minimum Inhibitory Concentrations (MICs) of the antibiotics, β-Lactamase production, conjugation and plasmid profile was studied on the bacterial isolates using standard microbiological protocols.

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Results: A total of 62 *E. coli* were isolated out of 200 door handles sampled and their susceptibilities to ten different commonly used antibiotics were determined. All the isolates had 87 – 100% resistance to all tested antibiotics with the highest susceptibility (13%) exhibited to only Gentamicin and Imipenem. Thirty-two of the isolates have Multiple-Antibiotic Resistance (MAR) index of 1.0 and 21(65.6%) of them produced β-lactamase enzymes. Thirteen (59.09%) of the multiple antibiotics resistant *E. coli* isolates transferred resistance plasmid to *Proteus mirabilis* via conjugation. Electrophoresis of plasmid DNA in the test multi-antibiotics resistant *E. coli* isolates showed varying number of plasmids with molecular weights ranging between 1200 and 3000 base pairs.

Conclusion: This study has showed that multi-antibiotic resistance genes from test *E. coli* could be transmitted to pathogenic bacteria which can result in serious health hazard. Thus, improved hygiene practices should be encouraged and constant microbiological surveillance of door handles in these higher institutions should be encouraged to determine effective antibiotics to solve the health hazard that may arise from *E. coli* infections.

Keywords: Escherichia coli; antibiotic susceptibility; plasmid profiles; door handles.

1. INTRODUCTION

The spread of infectious diseases through hand contact has been an area of major concern. According to a study conducted by Itah and Ben [1], Gram-positive *Staphylococcus aureus*, and Gram-negative enteric bacteria such as *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, were found to contaminate various contact surfaces including chairs, tables, windows, door handles, and many other common household fixtures. Infectious diseases top the list for causes of death worldwide and contribution to morbidity and mortality cannot be readily quantified due to lack of data for most countries and it remains a global concern [2].

Diseases commonly spread by means of environmental surfaces such as computers, classroom walls, door handles, toilets, chairs, and so on, include; the common cold, cold sores, conjunctivitis, giardiasis, impetigo, meningitis, pin worm disease, diarrhoea and pneumonia, to mention but a few. Bacteria such as *E. coli*, *Shigella dysenteriae*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Staphylococcus aureus* as well as *Corynebacterium diphtheria* causes diarrhoea, dysentery, pneumonia, food poisoning and intoxication as well as whooping cough respectively [3].

Human hands have been implicated as the major transmitter of microorganisms to environmental surfaces. Curtis and Carncross [4] reported that hands often act as vectors that carry disease-causing pathogens including bacteria and viruses from person to person either through direct contact or indirectly via surfaces. Defective personal hygiene can facilitate the transmission of some of these pathogenic bacteria found in the environment to human hands [5].

Some research results indicate that surfaces that are routinely touched with hands have higher bacteria load as compared to restroom floor and toilet seats. One could expect the opposite to be true. This observation could be due to cumulative contamination of door handles as result of poor sanitary conditions (not washing and cleaning hands with disinfectants after using the toilets) [6]. Hand washing which is traditional was the first line of defense in preventing the spread of disease; it has been neglected and must be embraced vigorously by families, schools and healthcare professionals. However many people seem to run water over their hands without using soap and some fail to wash their hands at all after leaving the restroom [7].

Bacteria are microscopic organism found everywhere in the universe as pathogenic or non-pathogenic. They are found in the environment all around us and within each one of us, there are trillions and trillions of them. Majority of them are harmless to human and animals but those few which are harmful can lead to death of affected individuals [8,9]. The time of survival depends on the type of pathogen, majority including S*higella*, *Escherichia*, *Clostridium*, severe acute respiratory syndrome (SARS) coronavirus, and norovirus which can survive on surfaces for weeks or even months [10].

E. coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms

(endotherms) [11]. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination [12]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin B_{12} and K [13], and preventing colonization of the intestine with pathogenic bacteria [14,15].

E. coli and other facultative anaerobes constitute about 0.1% of gut flora [16], and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination [17].

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa, Nasarawa State, Nigeria. Keffi is approximately 68 Km from Abuja, the Federal Capital Territory and 128 km from Lafia, the Capital of Nasarawa state. Keffi is located between latitude 8°5 N of the equator and longitude 7°8 E and situated on an altitude of 850 m above sea level and Nasarawa is approximately 35 km South-west from Keffi [18].

2.2 Sample Collection

Sterile swab sticks were used for the collection of the samples as described by Opere et al. [19] with some modification. The swab sticks were immersed in 0.85% sterile normal saline solution and each door handle was swabbed immediately with a single sterile swab stick, and replaced into its cover immediately. The samples were then transported to the microbiology laboratory of Nasarawa State University, Keffi for the analysis.

2.3 Isolation of *Escherichia coli*

The isolation of *E. coli* was carried out as described by Opere et al. [19] with some modifications; the swab sticks were inoculated aseptically into Bijou bottles containing sterile Nutrient Broth medium and incubated at 37℃ for 24 hours. Specimens from the Nutrient Broth medium were then sub-cultured by streaking on Levine Eosin Methylene Blue (EMB) Agar plates aseptically, using sterile wire loop and incubated at 37℃ for 24 hours. The plates were observed after 24 hours incubation, greenish metallic sheen indicates the presence of *E. coli*; further biochemical and immunological tests were performed to confirm the organism.

2.4 Antibiotics Susceptibility Test

The antibiotics susceptibility test of the isolates was carried out using Kirby-Baeur disk diffusion method with some modifications as described in Clinical Laboratory Standard Institute manual [20].

2.5 Determination of Minimum Inhibitory Concentrations

The Minimum Inhibitory Concentration (MIC) of six of the antibiotics used was studied using standard agar dilution method following the procedures described in the Clinical Laboratory Standard Institute Manual [20].

2.6 Detection of β-lactamase Enzymes Producing *E. coli*

Iodometric and acidometric methods were used in the detection of β-lactamase producing species of *E. coli* from selected multiple antibiotics resistant isolates using standard procedures as described by Samant and PAI [21].

2.7 Conjugation Experiment

The transfer of resistance traits by the resistant isolates of *E. coli* to ciprofloxacin sensitive *Proteus mirabilis* was investigated using the methods described by Onaolapo and Klemperer [22] with some modifications.

2.7.1 Curing transconjugants

The curing of transconjugants *P. mirabilis* (Rf plasmid) was carried out by treating the *P. mirabilis* transconjugants with acridine orange dye as described by Onaolapo and Klemperer [22].

2.8 Plasmid DNA Analysis of Isolates

2.8.1 Preparation and purification of total DNA using spin-column protocol

The transconjugant strains and resistant isolates were subjected to plasmid DNA isolation

following the protocol of Bimboim and Doly [23] and Vogelstein and Gillespie [24].

2.8.2 Detection of number and sizes of plasmid DNA (Agarose gel electrophoresis)

One per cent (1.0%) agarose gel was used to resolve DNA fragment. This was prepared by combining 1 g agarose in ten times concentration of tris-borate ethylene diamine tetraacetate (10 ml 10X TB-EDTA) buffer and 90 ml sterile distilled water in 250 ml beaker flask and heating in a microwave for 2 minutes until the agarose is dissolved [25].

Exactly 0.5 µl of Ethidium bromide was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and casting combs were inserted. This was allowed to gel for 30 minutes. The casting combs were carefully removed after the gel had solidified completely. One times concentration (1X) TBE buffer was added to the reservoir until it covered the agarose gel. Precisely 0.5 µl of gel tracking dye (bromophenol blue) was added to 20 µl of each sample with gentle mixing. The sample was loaded onto the wells of the gel at a concentration of 20 µl, the mini horizontal electrophoresis gel setup was covered and electrodes connected. Electrophoresis was carried out at 100-200 mA for one hour. At the completion of electrophoresis, the gel was removed from the buffer and viewed under UV-transilluminator. The band pattern of DNA fragments was photographed with a Polaroid camera and documented using electrophoresis gel documentation system.

2.9 Statistical Analysis

Statistical computation of data obtained was performed using Microsoft excel^{TM} 2010 and Smith's Statistical Package (SSP) version 2.8 for analysis Chi-square test and analysis of variance (ANOVA) were used to compare the results.

3. RESULTS AND DISCUSSION

3.1 Results

Of the 200 door handles examined, *E. coli* was isolated from 62 of the samples. A total number of 36 of the bacterial isolates were from Nasarawa State University, Keffi (NSUK) and 26 of the isolates were from Federal Polytechnic, Nasarawa (FPN) (Table 1). Distribution of *E. coli* in different location studied showed that; Faculty of Social Sciences in Nasarawa State University account for least prevalence of the bacterial isolates with 4 (20.00%) occurrence. Faculty of Arts 9 (45.00%) has the highest prevalence of the bacteria. Faculty of Administration and Faculty of law are both tied with 8 (40.00%), While Faculty of Natural and Applied Sciences Accounted for 7 (35.00%) of the prevalence (Fig. 1). Fig. 2 shows the distribution of *E. coli* isolates in different locations in Federal Polytechnic, Nasarawa, where School of Business Studies (50.00%) has the highest prevalence while School of Basic and Applied Sciences, School of Environmental Studies, School of Engineering Technology and School of General Studies has the prevalence rate of 30.00%, 25.00%, 20.00% and 5.00% respectively.

The antibiotic susceptibility testing of *E. coli* isolates showed that 8 (12.90%) of the isolates were susceptible to Gentamicin and Imipenem, 6 (9.68%) were susceptible to Co-trimoxazole and Nitrofurantoin. Ceftazidime was the least effective in all the antibiotics tested, with 100% resistance (Table 2). Susceptibility test results on the isolates from the two different institutions showed that Nitrofurantoin 5 (13.89%) was most effective at Nasarawa State University, Keffi, while Imipenem 6 (23.08%) was the most effective antibiotics in Federal Polytechnic, Nasarawa. Ceftazidime, Cefuroxime and Augmentin were found to be 100% inactive against isolates from Nasarawa State University, Keffi; while, Ceftazidime and Ciprofloxacin were also 100% resistant against the isolates from Federal Polytechnic, Nasarawa (Table 3).

The Multiple Antibiotics Resistance (MAR) Index was computed and it showed that most of the isolates had MAR Indices ≥ 0.2, which implies that they are resistant to two or more of the test antibiotics; whereby 35.48% of the isolates had MAR Index 0.1, meaning, they are susceptible to all but one of the test antibiotics; and 51.61% had MAR index 1.0 (Table 4).

Table 5 shows antibiotic susceptibility profile of 32 isolates based on Minimum Inhibitory Concentrations determined using EUCAST (2015). Peak plasma level of each tested antibiotics was used as breakpoint to determine resistance. Imipenem was observed to be the most effective test antibiotic with only 2 (6.25%) resistant test *E. coli* isolates. However, the *E. coli*

isolates were highly resistant to Augmentin 29 (90.63%) followed by Nitrofurantoin 27 (84.38%). This result shows that susceptibility to the antibiotics based on MIC and Peak plasma levels follows this order: Imipenem > Gentamicin > Ciprofloxacin > Co-trimoxazole > Nitrofurantoin > Augmentin.

Results from this study shows that Augmentin was 100% and 73% ineffective against the test bacterial isolates in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa respectively. There was no resistant *E. coli* isolate to imipenem in Nasarawa State University, Keffi while 18% Imipenem resistant *E. coli* isolates was observed from Federal Polytechnic, Nasarawa (Table 6).

Beta-lactamase enzyme production test was performed on 32 of the selected resistant *E. coli* isolates and 21 of the isolates shows positive for beta lactamase enzymes production. The result of this test is shown in Table 7.

The result of the conjugation studies on Table 8 showed that out of the twenty two (22) donor isolates, thirteen were observed to transfer resistance traits to *Proteus mirabilis*. The MICs of ciprofloxacin on transconjugants after conjugation was also observed to increase.

Changes were observed in the sensitivity pattern of tested transconjugants after curing. The MICs of ciprofloxacin for each transconjugants decreased significantly when compared to those before curing (Table 9).

Agarose gel electrophoresis was used to determine the number and molecular weight of plasmid DNA in some selected isolates that were observed to have transferred resistance to a non-resistant *P. mirabilis* in conjugation experiment. This analysis showed that all the resistant isolates harbours varying number of plasmids of various molecular sizes ranging from 1200 to 3000 base pairs (Plate 1).

Table 1. Distribution of *E. coli* in door handles of Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa

Institution	No. of samples	No. of isolates (%)
NSUK	100	36 (36.00)
FPN	100	26 (26.00)
Total	200	62(31.00)

$\chi^2 = 0.5467$
P = 0.4596
Key: NSUK –Nasarawa State University, Keffi
FPN –Federal Polytechnic, Nasarawa

3.2 Discussion

Microbiological assessment of door handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa showed 31.00% contamination rate of the door handles with *E. coli*. This high level of bacterial contamination of door handles could be a consequence of poor hygiene practices within the institutions. Also, the isolation rate between the two institutions showed no statistical significance.

The number of *E. coli* isolated in this study is in consonance with other studies involving isolation of *E. coli* from door handles, crevices, bannisters, toilet knobs and other handy surfaces both internationally and locally [6,26,27,28,29].

Fig. 1. Distribution of *E. coli* in door handles from different locations in Nasarawa State University, Keffi
KEY: FNAS– Faculty of Natural and Applied Sciences, FARTS– Faculty of Arts, FADM– Faculty of Administration, FSOC– Faculty of Social Sciences, FLAW– Faculty of Law
$\chi^2 = 1.2922$, P = 0.8627

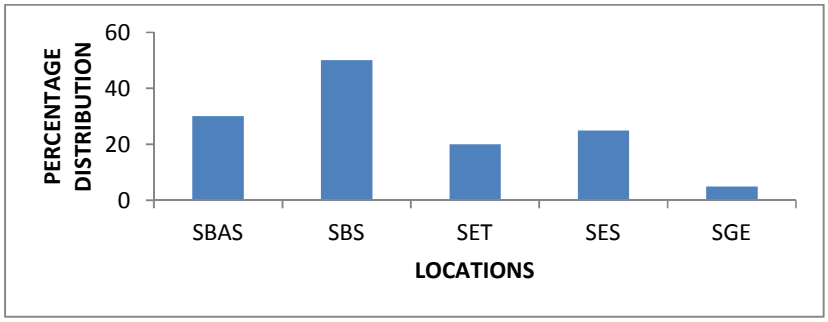

Fig. 2. Distribution of *E. coli* in door handles from different locations in Federal Polytechnic, Nasarawa

KEY: SBAS –School of Basic and Applied Sciences, SBS –School of Business Studies, SET –School of Engineering Technology, SES –School of Environmental Studies, SGE–School of General Studies,
χ^2 = 0.8714, P = 0.9286

Table 2. Susceptibility profile of *Escherichia coli* isolates from door handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa

Antibiotics	Susceptibility (%) (n=62)
Ceftazidime	0(0.00)
Cefuroxime	3(4.84)
Gentamicin	8(12.90)
Ciprofloxacin	1(1.61)
Ofloxacin	5(8.06)
Augmentin	1(1.61)
Nitrofurantoin	6(9.68)
Ampicillin	4(6.45)
Sulphamethoxazole/ Trimethoprim	6(9.68)
Imipenem	8(12.90)

Table 3. Susceptibility profile of *E. coli* from door handles in the two different institutions studied

Antibiotics	No. susceptible (%)	
	NSUK (n=36)	FPN (n=26)
Ceftazidime	0(0.00)	0(0.00)
Cefuroxime	0(0.00)	3(11.54)
Gentamicin	3(8.33)	5(19.23)
Ciprofloxacin	1(2.78)	0(0.00)
Ofloxacin	2(5.56)	3(11.54)
Augmentin	0(0.00)	1(3.85)
Nitrofurantoin	5(13.89)	1(3.85)
Ampicillin	3(8.33)	1(3.85)
Sulphamethoxazole/ Trimethoprim	3(8.33)	3(11.54)
Imipenem	2(5.56)	6(23.08)

Key: NSUK –Nasarawa State University, Keffi, FPN –Federal Polytechnic, Nasarawa

Table 4. Multiple Antibiotics Resistance (MAR) index of *E. coli* from Door Handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa

MAR index	No. of isolates (n=62)	Percentage
0.10	22	35.48
0.20	6	9.68
0.30	2	3.23
1.00	32	51.61

Table 5. Antibiotics susceptibility profiles of selected isolates based on their M.I.C and peak plasma levels

Antibiotics	Peak plasma level (µg/ml)	No. (%) resistant (n= 32)
Gentamicin	10.0	6(18.75)
Ciprofloxacin	4.4	10(31.25)
Augmentin	5.0	29(90.63)
Nitrofurantoin	64.0	27(84.38)
Co-trimoxazole	5.0	22(68.75)
Imipenem	14.0	2(6.25)

The presence of *E. coli* isolated in door handles studied indicates possible faecal contamination. There is a possibility of contamination with other enterobacteriaceae such as *Enterobacter*, *Salmonella*, *Proteus*, *Klebsilla*, *Citrobacter*, *Yersinia* and *Providencia* [30]. Organisms from the enterobacteriaceae group have been isolated from door handle and other surfaces [26,28,29]. *E. coli* have been reportedly linked to diarrhoeal diseases, urethrocystitis, prostatitis and pyelonephritis [31,32].

Being enteric bacteria, the presence of *E. coli* indicates poor hygiene practices among students and staff of the institutions such as, not washing and cleaning hands with disinfectant after using toilets [6]. Hand washing has been traditionally first line defense in preventing diseases [7].

Table 6. Resistance profile of selected isolates from the two institutions studied based on MIC and peak plasma levels

Antibiotics	Peak plasma level (µg/mL)	No. (%) resistant	
		NSUK (n=21)	FPN (n=11)
Gentamicin	10.0	2(9.52)	3(27.27)
Ciprofloxacin	4.4	7(33.33)	3(27.27)
Augmentin	5.0	21(100.00)	8(72.72)
Nitrofurantoin	64.0	18(85.71)	9(81.81)
Co-trimoxazole	5.0	14(66.67)	8(72.72)
Imipenem	14.0	0(0.00)	2(18.18)

Key: NSUK –Nasarawa State University, Keffi, FPN –Federal Polytechnic, Nasarawa

Antibiotics susceptibility assessment of *E. coli* isolates from door handles in the two institutions showed varying degrees of antimicrobial resistance as well as multiple antibiotics resistances to the antibiotics tested. Result of antibiotic susceptibility tests on the bacterial isolates revealed that most of the isolates were multi-antibiotics resistant to more than three of the antibiotics as also reported in other studies on door handles and other public surfaces [26]. This observation suggests that the isolates in this study may probably have originated from an environment where antibiotics are often used indiscriminately [33]. Broad-spectrum antibiotics are sometimes reported to be given in place of narrow-spectrum antibiotics as a substitute for culture and sensitivity testing, with the consequent risk of selection of antibiotic-resistant mutants [34,35,36].

The order of antibacterial ineffectiveness of the studied antibiotics generally was Ceftazidime (100%) > Augmentin (98.39%) = Ciprofloxacin (98.39%) > Cefuroxime (95.16%) > Ampicillin (93.55%) > Ofloxacin (91.94%) > Co-trimoxazole (90.32%) = Nitrofurantoin (90.32%) > Gentamicin (87.10%) = Imipenem (87.10%). Gentamicin 8 (12.90%) and Imipenem 8 (12.90%) were the most effective of all the antibiotics tested, this could be as a result of accessibility to the antibiotics and the parenteral routes of administering them.

The findings from this present study agrees with the work reported by Okonko et al. [37], who reported high bacterial isolates resistance to ampicillin, augmentin and co-trimoxazole (60 to 100%). Other researchers have reported bacterial isolates obtained from public surfaces to be resistant to co-trimoxazole [26,27,28,29]. This study highlights a highly diverse antibiotics resistance rates among the bacterial isolates. Antibiotic resistance of isolated bacteria from door handles may be a reflection of the harmful effects of self-medication.

Table 7. Beta lactamase enzymes production in selected *E. coli* isolates

Isolates	Acidometric	Iodometric
FNAS 02	+	+
FARTS 01	+	+
FARTS 02	+	+
FARTS 08	+	+
FARTS 13	-	-
FARTS 17	+	+
FARTS 18	+	+
FARTS 19	+	+
FADM 12	+	+
FADM 14	-	-
FADM 19	+	+
FSOC 04	+	+
FSOC 07	+	+
FSOC 09	+	+
FSOC 12	-	-
FLAW 09	-	-
FLAW 10	-	-
FLAW 11	+	+
FLAW 12	-	-
FLAW 19	+	+
FLAW 20	+	+
FPN 03	-	-
FPN 15	-	-
FPN 17	-	-
FPN 18	+	+
FPN 26	+	+
FPN 38	+	+
FPN 49	-	-
FPN 50	+	+
FPN 57	+	+
FPN 79	+	+
FPN 86	-	-

The multiple antibiotic resistance indices (MARI) offers an indirect suggestion of the probable sources of an organism. According to previous researchers, Krumperman [34] and Paul et al. [33], MAR index greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used. Multi-antibiotic resistance of three to eight

antibiotics was frequently observed in this study among the *E. coli* isolates. Out of sixty two *E. coli* studied, 51.61% had MAR index of 1.0, while 35.48% had MAR index of 0.1; 9.68% had MAR index of 0.2 and 3.23% had MAR index of 0.3. Such multi-antibiotic resistance has important implications for the empiric therapy of infections caused by *E. coli* and other enterobacteriaceae, and for the possible co-selection of antibiotic resistance mediated by multi-antibiotic resistance plasmids [38,39]. It has been well documented that gram negative bacilli habours series of antibiotic resistance genes like transposons or integrons and R plasmids which can be transferred to other bacteria horizontally [40,41,42,43].

Table 8. Minimum inhibitory concentration (M.I.C) of ciprofloxacin before and after conjugation

Isolates	MIC before conjugation (µg/mL)	Recipient characteristics	MIC of transconjugants (µg/mL)
FARTS 02	4.00	+	4.00
FARTS 13	0.25	+	2.00
FARTS 17	8.00	+	8.00
FARTS 18	8.00	-	-
FADM 12	8.00	-	-
FADM 19	4.00	+	8.00
FSOC 04	8.00	-	-
FSOC 07	8.00	+	8.00
FSOC 09	8.00	+	16.00
FSOC 12	0.50	+	8.00
FLAW 09	8.00	-	-
FLAW 10	0.25	+	4.00
FLAW 11	0.50	+	2.00
FLAW 19	4.00	+	8.00
FLAW 20	2.00	-	-
FPN 18	8.00	+	8.00
FPN 26	4.00	+	4.00
FPN 49	1.00	-	-
FPN 50	8.00	-	-
FPN 57	8.00	-	-
FPN 79	4.00	+	8.00
FPN 86	0.50	-	-

**MIC of Ciprofloxacin on recipient Proteus mirabilis before conjugation = 0.125 µg/Ml*
+ Represents isolate showing pink on MacConkey agar plates.
- Represent isolates that did not transfer resistant traits

Table 9. Minimum inhibitory concentrations (M.I.C.) of ciprofloxacin on transconjugants

Donor isolates code	MIC before curing (µg/mL)	MIC after curing (µg/mL)
FARTS 02	4.00	2.00
FARTS 13	2.00	0.50
FARTS 17	8.00	4.00
FADM 19	8.00	8.00
FSOC 07	8.00	4.00
FSOC 09	16.00	8.00
FSOC 12	8.00	0.50
FLAW 10	4.00	1.00
FLAW 11	2.00	0.25
FLAW 19	8.00	4.00
FPN 18	8.00	8.00
FPN 26	4.00	0.25
FPN 79	8.00	4.00

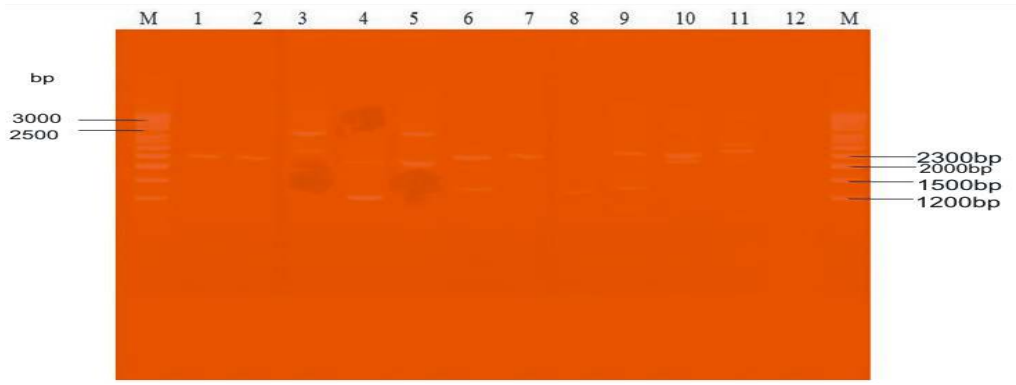

Plate 1. 1% Agarose gel electrophoresis of plasmid DNA from multiple antibiotic resistant *Escherichia coli* isolates and the recipient *Proteus mirabilis* before conjugation

Lane M: Supercoiled DNA Ladder (Marker) composed of DNA fragments (in base pairs).
Lane 1 to 11: Resistant Escherichia coli (Lab numbers; FARTS02, FARTS17, FADM19, FSOC07, FSOC09, FSOC12, FLAW10, FLAW11, FLAW19, FPN18, FPN26 respectively).
Lane 12: Recipient Proteus mirabilis before conjugation

Beta-lactamase production investigation revealed that twenty-one out of the thirty-two resistant *E. coli* isolates from door handles tested for ß-lactamase enzyme in this study produced beta-lactamase enzyme capable of hydrolysing beta lactam antibiotics. Akpan [44] also reported similar result in Nigeria. This observation confirmed the high beta-lactam antibiotics resistance that was observed against ampicillin, augmentin, ceftazidime and cefuroxime. The implication of these resistances is that many bacterial diseases that could be treated with inexpensive antibiotics, has recently been made more expensive and less successful by the emergence and spread of resistant organisms [37,45]. However, these multi-antibiotic resistances observed among some of the bacteria isolates from door handles in this study has now become a large and growing problem in infections that account for most of Africa's disease burden, including respiratory and diarrhoeal diseases [45].

Resistance genes are often located on extra-chromosomal genetic elements or in segments inserted within the chromosome that originates from other genomes [46,47]. The acquisition of a new gene may occur by genetic transformation or through mobilization by conjugative transfer. The latter may occur at high frequency and efficiency, and several resistance genes can be acquired simultaneously [46]. Plasmid profiles have been reported to be useful in tracing the epidemiology of antibiotic resistance. The result of the conjugation studies suggested possible acquisition of R-plasmids by sensitive *P. mirabilis* from multiple antibiotic resistant isolates. It was observed that out of twenty two donor *E. coli* isolates, thirteen transferred resistance traits to ciprofloxacin sensitive *P. mirabilis* (Table 8). The result of antibiotics susceptibility of the transconjugants using M.I.C method was seen to have changed after conjugation. There was an increase in the M.I.Cs of the bacterial isolates. Changes were observed in the sensitivity pattern of tested transconjugants after curing with acridine dye. Decrease in minimum inhibitory concentration of transconjugants after curing as compared to those before curing revealed that acridine dye was effective curing agent. However, conjugation analysis revealed that apart from plasmids that were transferable by conjugation, other resistance determinants were transferable to sensitive recipient strain of *P. mirabilis* since their M.I.Cs increased. This suggests that these resistance determinants were carried extra-chromosomally on R-plasmids. Similar resistance determinants movement have been attributed to the selection pressure created by uncontrolled use of antibiotics in feed-stuff for animals, in addition to the unregulated use of antibiotics by humans [48,49,50]. Indiscriminate use of antibiotic agents and antibiotic sale behaviour (for example, sale of antibiotics without prescription, sale of under dose and substituting brands) has been reported to enhance the development of antibiotic resistance among pathogenic bacteria. In developed countries, the main reservoirs for antibiotic resistance in enteric bacteria have been attributed to farm animals such as cattle, sheep, pigs and poultry [51,52]. Contact with

these animals or consumption of food products from them such as milk has been the main route of dissemination of resistance into the human populations.

Agarose gel electrophoresis analysis showed the presence of plasmid of various sizes among the multiple antibiotics resistant isolates ranging from 1200 – 3000 base pairs. All the corresponding transconjugants contained similar plasmid sizes.

The plasmid profiles observed in this study indicated that the plasmids are distributed at random in these isolates. In most of the cases, bacterial isolates having similar antibiotic sensitivity patterns had different plasmid patterns. According to some researchers such as Carattoli [46] and Yah et al. [47], antibiotic resistance in some bacterial isolates which seem not to possess plasmids was associated with chromosome and/or transposons. In determining whether the plasmids resistance markers could be transferred to sensitive isolates, the results showed that all the transconjugants expressed plasmid DNA that migrated approximately on agarose gels. All the *E. coli* isolates examined in this study had MAR Index of 1.0, meaning, they are resistant to all the 10 antibiotics tested. This shows that there is a relationship between possession of plasmid and resistance to antibiotics.

Multiple resistance genes clusters in large plasmids are usually associated with transposons and insertion sequences [53]. Plasmid profiles revealed that bacterial isolates with the same resistance profile may differ in their plasmid profiles. This suggests diversity in plasmid contents of bacterial isolates and the contribution of different plasmids in the resistance to a certain antibiotic. The exchange of plasmids between bacterial cells and the integration of resistance genes into specialized genetic elements play a major role in acquisition and dissemination of antibiotic resistance genes among bacteria isolates [46,47,54,55,56].

4. CONCLUSION

a. Findings from this research showed that up to 32% of door handles in Nasarawa State University, Keffi and Federal Polytechnic, Nasarawa is contaminated with *E. coli*.
b. The *Escherichia coli* isolates were generally resistant to test antibiotics in the order of Ceftazidime (100%) > Augmentin (98%) = Ciprofloxacin (98%) > Cefuroxime (95%) > Ampicillin (94%) > Ofloxacin (92%) > Co-trimoxazole (90%) = Nitrofurantoin (90%) > Gentamicin (87%) = Imipenem (87%).
c. Fourteen out of sixty-two isolates are shown to have transferred resistance factors to non-resistant bacteria (*Proteus mirabilis*) through conjugation experiment.
d. Agarose gel electrophoresis reveals that all the donors and the transconjugants tested harbours at least one or two plasmids of different molecular weights between 1200 to 3000 base pairs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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